rabbits were kept on a strictly controlled diet for 48 hours prior to the injection of epinephrine, and the phenolic content of the urine was determined in a 24-hour specimen. An increase in phenolic substances was obtained after epinephrine injection equivalent to 80 per cent. of the injected drug. A portion of the urine was acidified with acetic acid and allowed to stand for 48 hours, filtered, hydrolyzed by NaOH and concentrated under reduced pressure. The residue was extracted several times with 90 per cent. alcohol, and the extract reduced to dryness. The dark brown mass was extracted with ether. Upon evaporating the ether extract, a small quantity of crystalline material was obtained which gave characteristic tests for protocatechuic acid. The amount of material isolated was too small for combustion analysis. It would appear highly probable that protocatechuic acid may be an end product of epinephrine, since injected protocatechuic acid is excreted partly unchanged and partly as an ethereal sulfate.[7] Furthermore, Dakin[8] showed that phenyl serine and phenyl-glyceric acid are oxidized to benzoic acid and that p-hydroxy proprionic acid is oxidized to p-hydroxy benzoic acid. Comparing these structures with that of epinephrine, it is conceivable that a similar oxidation process might take place in the animal body yielding protocatechuic acid.

As an alternative hypothesis for the rapid disappearance of epinephrine from the blood, the following may be suggested from a critical consideration of the present findings; epinephrine is not destroyed by the blood nor to any significant extent by specific organs, but passes rapidly through the capillaries into the tissues, where it is oxidized to a physiologically inactive substance, possibly protocatechuic acid.

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MENINGOCOCCUS INFECTION OF THE CHICK EMBRYO[1]

GOODPASTURE and Anderson[2] have recently reported on the use of the chick embryo in the study of infections by various types of bacteria. They followed the technique previously described by Goodpasture and Buddingh[3] for the cultivation of vaccinia virus. This method offers a distinct advantage in that the behavior of infectious agents can be studied *in vivo* in a uniform sterile living culture medium. Observations on some of the phenomena of invasion and the earlier stages in the pathogenesis of various infections can be made with comparative ease and simplicity. Furthermore, the possibility of cultivating microorganisms which heretofore have been maintained with difficulty on artificial media or in laboratory animals presents itself.

In this communication we wish to report our findings on the cultivation of the *Micrococcus meningitidis* by this method.

A pure culture possessing all the staining and morphological characteristics of *Micrococcus meningitidis* was obtained directly from the spinal fluid of a patient with the typical clinical picture of cerebrospinal meningitis. Fermentation reactions were typical; it was agglutinated by a polyvalent antimeningococcus serum and the Type I monovalent serum (Gordon's classification) in dilutions of 1–100. No agglutination took place in normal horse serum.

The microorganism from the first culture obtained directly from the spinal fluid was inoculated onto the chorio-allantoic membrane of chick embryos twelve days old. A platinum loopful of the 18-hour blood agar slant culture was used as inoculum. At the end of 24 hours the majority of the embryos had died from the infection. In the remaining ones, Gram-negative diplococci in large numbers, both intra- and extracellular, could be demonstrated by smears made from the membranal exudate. Transfers by a platinum loopful of membranal exudate to fresh 11- or 12-day-old embryos have been made every 24 hours. The purity of the culture has been controlled by stained smears and inoculation of the membranal exudate on blood agar slants. Agglutination and fermentation reactions have been set up at frequent intervals. In this manner the strain has been passed through 100 serial transfers in the chick embryo without loss of its type specificity.

Throughout this period of investigation the infection has been uniformly lethal for the chick embryo. Death usually occurred 24 to 48 hours after inoculation. Cultures from the heart's blood of the embryo were usually positive for the meningococcus, indicating that, besides infection of the membrane, the embryo itself is also invaded.

A histological study of the lesion in the chorio-allantoic membrane and the chick embryo was undertaken. Twenty-four- and 48-hour membranal lesions and embryos were fixed in Zenker's (10 per cent. acetic acid) and embedded in paraffin. Sections were stained with hematoxylin and eosin and by Giemsa's method to demonstrate microorganisms.

Grossly the membranal lesion is not very striking. Twenty-four hours following inoculation there is slight cloudiness and swelling flecked with small streaks and patches of hemorrhage. Microscopically,

[7] E. Baumann, *Zeits. physiol. Chem.*, 1: 263, 1877.
[8] H. D. Dakin, "Oxidation and Reduction in the Animal Body," Longmans, Green and Company, 1922.
[1] Aided by a grant from the Division of Medical Sciences, Rockefeller Foundation.
[2] E. W. Goodpasture and K. Anderson, *Am. Jour. Path.*, 13: 149, 1937.
[3] E. W. Goodpasture and G. J. Buddingh, *Am. Jour. Hyg.*, 21: 319, 1935.

there is edema and a slight cellular infiltration with polymorphonuclears and monocytes of the mesoderm. Most striking are the numerous extensive hemorrhages within this layer. The ectodermal layer is usually covered with more or less cellular exudate in which the microorganism is present in large numbers. Necrosis of this layer does not occur except in those areas in which the blood supply in the underlying mesoderm is interfered with by hemorrhage or occlusion of the vessels. The most striking lesions are found in and around the blood vessels. The meningococcus invades the mesoderm and is found most abundantly in the areas of hemorrhage and in the lumen of the vessels. That a special affinity for the endothelial cells of the vessels obtains is evident from the fact that the microorganism is usually found in great numbers on the surface of these cells, often forming a complete collar around the inner surface of the vessel. It does not apparently grow intracellularly. Swelling and necrosis of vascular endothelium, with subsequent hemorrhage or thrombosis of the smaller vessels, result. The endodermal layer of the membrane is not greatly affected by the infection.

Within the embryo proper the microorganism produces lesions, particularly in the heart, meninges, kidneys and skin. All the lesions are vascular in origin and are evidently initiated by the lodging of the microorganism on the endothelium of the capillaries, with a resulting hemorrhage or thrombosis. In the heart numerous scattered foci of necrosis are found around small vessels and capillaries in which the diplococci can be demonstrated in close association with the endothelial cells. The meninges and choroid plexus show small areas of hemorrhage from small vessels in and around which numerous typical microorganisms can be found. The small capillaries of the kidney glomeruli are usually plugged with diplococci, so much so that an entire glomerulus often appears as a deep blue staining mass. In the skin and subcutaneous tissue hemorrhages are particularly frequent. The microorganisms are found here in abundance, growing on endothelial cells and among the escaped red blood cells. Vascular lesions of this type are occasionally observed in striated muscle, bone marrow and submucous tissues of the pharynx. In a few cases where multiplication of the microorganism within the embryo is particularly abundant the Kupffer cells of the liver are loaded with them.

Although these findings do not warrant any definite conclusion as to the pathogenesis of the earlier stages in the infection with the *Micrococcus meningitidis* in the human, it is well recognized that the purulent meningitis found at autopsy is an end stage in a disease which in its earlier stages is characterized by numerous pathological changes elsewhere in the body. The presence of the meningococci in the blood stream, sometimes as a chronic bacteremia, and the commonly recognized purpuric hemorrhages in the skin from which the microorganisms have been recovered by many observers indicate that the meninges may be secondarily invaded by meningococci transported by the blood stream.

In the experimental infection of the chick embryo the affinity of the meningococcus, once it has invaded the embryonic membrane, for the blood and vascular endothelium is the most striking feature. The ensuing lesions are all the direct result of this particular circumstance. The infection is essentially a septicemia.

Up to the present the study of infection by the *Micrococcus meningitidis* has been greatly hampered in that no suitable laboratory animal has been available in which the microorganism could be propagated in series away from its human host. Its relatively low pathogenicity has made necessary the use of exceptionally large doses in order to produce lethal effects in guinea pigs and mice. The clinical and pathological picture of cerebro-spinal meningitis has been obtained in monkeys only irregularly after direct inoculation of the central nervous system with large numbers of microorganisms.

Propagation of the *Micrococcus meningitidis* in the chick embryo is of additional interest because of the possibility of using this method for analyzing the effect of anti-sera and anti-toxins upon the infection. These and other immunological problems provide a wide field for investigation. They are now being studied, and our findings will be reported at a later date.

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ENVIRONMENTAL CONDITIONS INFLUENCING THE DEVELOPMENT OF TOMATO POCKETS OR PUFFS

THE condition known as tomato "pockets" or "puffs" is a serious disease or abnormal condition of the fruit of this crop in the Atlantic and Gulf Coast States, and frequently in California. This abnormal condition of the fruit is more prevalent in the mid-winter and early spring crops grown in Florida, and especially in the early spring crops grown in Mississippi and Texas. Frequently 15 per cent. of the total crop is lost in Texas and often individual growers will lose as much as 65 per cent. of their crop. The malady is also of frequent occurrence on tomato crops grown in greenhouses in the north.

During the past five years an intensive study of the